TECHNICAL NOTES

Removal of Residual Protein from Cheese Whey Permeates by Bentonite

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ABSTRACT

Removal of residual protein from lactase-hydrolyzed cheese whey permeate by bentonite was affected by a variety of process components. Most efficient protein removal was by stirring presoaked bentonite with permeate (6.7% solids) at pH 4.5. At equivalent ash and pH, a greater percentage of protein was removed by bentonite from sweet whey permeate than from cottage cheese whey permeate.

INTRODUCTION

Attempts to purify sugar sirups from hydrolyzed lactose (HL) sweet whey permeate by ion exchange and heating for 10 min at 92 C and pH 4.5 resulted in troublesome foaming when the eluates were evaporated because only 45 to 55% protein was removed (4). Bentonite then was used to remove protein. Cerbulis (3) reported that with the addition of 3% bentonite (6 g/g protein) all of the protein was removed from cottage cheese whey at pH 4.6. Bentonite can clarify recalcitrant wines as well as remove protein (1). Larson and Yang (5) reported that the addition of .2 to .5% bentonite to Cheddar cheese whey permitted production of a low-protein clear wine. Dairy cows fed rations containing 5 to 10% bentonite increased fat and milk yields (8).

This paper discusses various factors affecting the removal of protein from HL sweet whey permeate by bentonite. These include the amount, type, and conditioning of the bentonite; pH, temperature, and total solids (TS) of the whey permeate; and stirring times and temperatures. Amounts of bentonite needed to remove protein from sweet and acid whey permeates are compared.

MATERIALS AND METHODS

Permeates

Sweet whey permeate containing 90% HL was processed from whole milk as described by Guy (4); HL cottage cheese whey permeate was processed similarly from skim milk. These permeates contained about 15% of the original protein content of the wheys, higher than may be encountered when a highly efficient ultrafiltration unit is used. The sweet whey permeate was condensed from 6 to 56% TS and frozen for storage; acid whey permeate was frozen at 5.5% TS. Sweet whey permeates with desired TS were prepared from the condensed permeate and were clarified by filtration.

Bentonite

Both Volclay custom granular and powdered sodium bentonite (American Colloid Co.)[2] were used for protein removal. Unless otherwise specified, wheys were held at 25 to 30 C for protein removal.

Removal of Ash by Ion Exchange Resins

Sweet whey permeate (22% TS) was demineralized by gravity flow thru a column of Amberlite 252 resin (H^+, 100 mesh, 4.5 x 30 cm) followed by passage thru Dowex 2 x 8 resin (OH^-, 25 to 50 mesh, 4.5 x 30 cm). This treatment yielded an eluate of 17.7% TS which was essentially ash-free, as indicated by a 98% decrease in conductance, and contained 70% less NPN without loss of initial protein content.

Protein Determination

Total nitrogen (TN) and nonprotein nitrogen (NPN) were determined by the Rowland method (7) with the micro-Kjeldahl procedure (2), and the difference between the two deter-

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[2] Reference to brand or firm name does not constitute endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

TABLE 1. Analysis of whey permeates.

Permeate	% Total solids	% Protein	% NPN X 6.38	% Ash
Sweet whey	6.7	.100	.197	.58
Cottage cheese whey	5.45	.076	.192	.62

minations was calcualted as protein nitrogen (PN). The residual protein contents of bentonite-treated and filtered permeates were determined nephelometrically at 650 mμ in 15% trichloracetic acid (TCA), (2 ml 50% wt/wt TCA of 1.257 specific gravity plus 6.0 ml of diluted permeate). Nephelometric determinations in 12% TCA were by adding 1.6 ml 50% TCA to 6.4 ml of diluted permeate. Optical densities through a 1 cm light path were read exactly 5 min after TCA was added to the clear filtrates.

Permeates of 20% TS treated with bentonite were diluted on a weight basis of 1:1 with water for nephelometric protein analysis by use of a standard curve constructed with a 10% total solids permeate. Permeates with lower TS concentrations were analyzed with their respective TS concentrations as standards.

Miscellaneous Methods

Ash was determined by the standard method for milk (2). Total solids were by the Mojonnier method (6). The concentrate on a weight to weight basis was diluted with distilled water to obtain lower total solids. Extent of lactose hydrolysis was determined with a Leeds and Northrup Enzymax Lactose/Glucose Analyzer.

Removal of Protein by Bentonite

Bentonite was added to 100 or 200 g of permeate either directly or after being presoaked 1 h in 10 parts of water. The pH then was adjusted with 2 N HCl, and the contents were stirred with a magnetic stirrer for 50 min at a 5 setting (medium speed). Samples were filtered clear by suction through wet Celite filter-aid and Whatman #1 filter paper. The first portion was discarded to prevent dilution.

RESULTS AND DISCUSSION

Both sweet and acid whey permeates contained comparable ash and NPN (Table 1). Linear absorption curves of turbidity relative to permeate concentration (protein concentration) up to .40 optical density (OD) were obtained with whey permeate in 15% TCA (Figure 1). Since concentrations of protein sufficient to produce OD above .40 gave nonlinear responses due to non-uniform aggregation of precipitated protein, suitable dilutions of permeate were made to keep the OD below .40. Since aggregation was time dependent, standards and test solutions were held exactly 5 min before OD was measured. As little as .03 to .05 mg protein N was determined by this technique.

Protein amounts with 15% TCA agreed more closely with Kjeldahl than those with 12% TCA

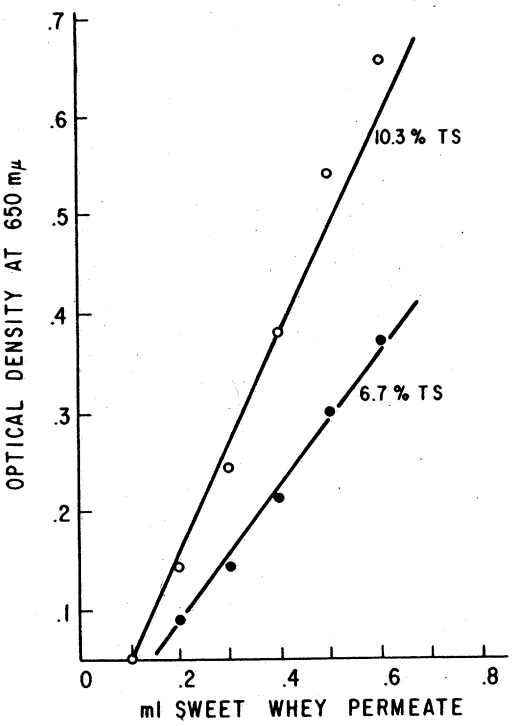

FIG. 1. Standard absorbance curves of sweet whey permeate in 15% TCA - ml of permeate to 8 ml total dilution. 6.7% TS - .157 mg N/ml.

TABLE 2. Percent protein removal by powdered bentonite in 6.7% total solids sweet whey permeate as measured by three procedures.

g Bentonite/ g protein at pH 4.5	% Protein removed		
		Turbidimetric	
	Micro-Kjeldahl	12% TCA	15% TCA
4.00	98.6	98.5	97.1
2.25	80.5	81.5	77.8
1.25	51.4	62.2	52.5
1.11	41.4	52.0	44.0
1.00	37.3	48.0	40.1

(Table 2). The NPN content was not affected by the direct addition of up to 4.0 g bentonite/g protein (.4% bentonite).

Maximum and equivalent amounts of protein were absorbed by either form of presoaked bentonite after being stirred for 50 min (Table 3). Direct addition of granular bentonite to sweet whey permeate removed less protein than powdered bentonite because of the lower surface area of the former. Presoaking powdered bentonite for 1 h in 10 parts of water doubled its efficiency of protein removal. Varying stirring speeds from slow to fast had no significant effect on protein removal. As the temperature of the permeate was increased from 12 C to 46 C, slightly more protein was removed (Table 4).

Optimum pH for protein removal was inversely proportional to the TS concentration of the permeate (Figure 2). The pH optimum was between 4.0 to 4.5 for 6.7% or 5.0% TS permeates, 3.0 to 4.0 for 10% TS, and 3.0 or less for a 20% TS permeate.

As the solids of the permeate were increased, more bentonite/g calculated protein at pH 4.5 was required to remove protein, especially when the powdered bentonite was added directly to the permeate (Figure 3). A diminerralized sample of 17.7% TS required the lowest bentonite/protein ratio for effective protein removal, implicating the ash content as controlling protein absorption. Presoaking the bentonite in water increased its efficiency for protein removal and greatly lessened the effects

TABLE 3. Effects of stirring at 25 C and conditioning of bentonite on protein removal from 10% total solids sweet whey permeate adjusted to pH 4.5.

Minutes stirring time	% Protein removed by 1.5 g bentonite/g protein		
	Powdered bentonite		Granular bentonite
	Add directly	Presoak 1 h 1:10 water	Presoak 1 h 1:10 water
5	....	....	78.6
10	....	....	80.0
15	42.0	83.0	85.0
30	42.0	85.5	85.0
50	45.0	86.0	86.5 (27.0)[a]
70	....	....	87.0
90	....	....	86.0

[a]Not presoaked.

TABLE 4. Effect of the temperature of 10% total solids sweet whey permeate (pH 4.5) on the extent of protein removal by 1.5 g of presoaked granular bentonite/g protein.

Temperature C	% Protein removed
12	82.2
25	85.2
38	89.2
46	92.0
55	88.3

of solids concentration on protein removal. These differences can be explained by the fact that presoaking the bentonite in water caused it to swell, thereby increasing its surface area, while adding bentonite directly to increasing concentrations of permeate progressively inhibited swelling. Once the bentonite swelled in water, addition of the permeate had only a slight effect on suppressing swelling.

More bentonite was required for protein

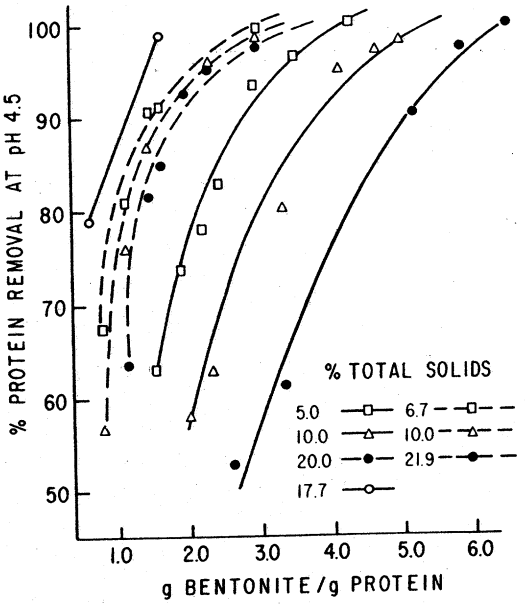

FIG. 3. Effect of total solids of sweet whey permeate on percent protein removal by powdered bentonite at pH 4.5. Dotted lines, presoaked bentonite; solid lines, bentonite added directly. o—o 17.7% total solids demineralized permeate.

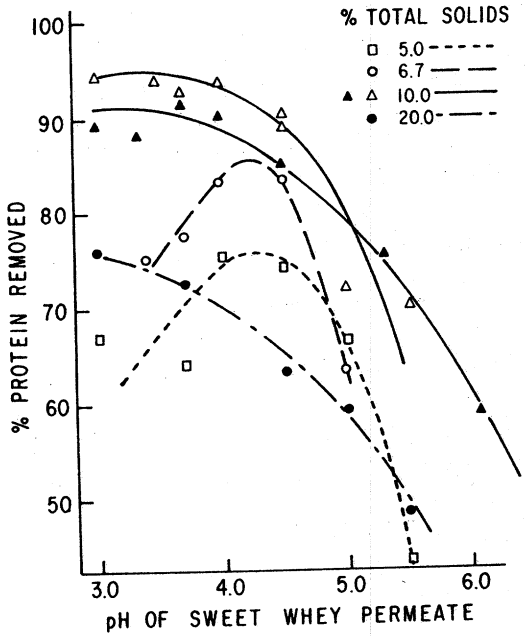

FIG. 2. Effect of pH of sweet whey permeate on percent protein removal by direct addition of powdered bentonite. □—□ 1.6, o—o 2.45, △—△ 3.23, and ●—● 3.5 g bentonite/g protein; ▲—▲ 1.5 g presoaked granular bentonite/g protein.

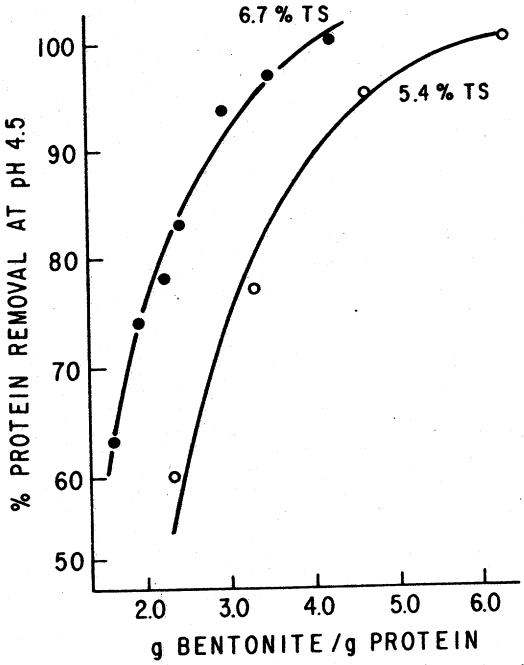

FIG. 4. Comparison of the amount of powdered bentonite required to remove protein from cottage cheese o—o and sweet whey permeates ●—● at pH 4.5.

removal from cottage cheese whey permeate than from sweet whey permeate (Figure 4), even though both were adjusted to pH 4.5 and had comparable ash contents; most probably differences in the Ca or P in the ash were responsible.

CONCLUSION

Bentonite can be used for the complete removal of protein from whey permeates. The protein involved does not require large amounts of bentonite. The bentonite-protein complex which forms settles readily and can be recovered by filtration or centrifugation after removal of the supernatant by siphoning. The bentonite, containing high quality protein, should be suitable for feed rations.

ACKNOWLEDGMENT

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